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EFFECTS OF SIMULATED ACID RAIN ON THE POLLEN GERMINATION AND POLLEN TUBE GROWTH OF APPLE (*MALUS SYLVESTRIS* MILLER CV. GOLDEN)

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The pollens of apple flowers have been treated with simulated acid rain solutions in range of pHs 2.9 to 5.0 in order to determine the threshold proportion values that lead the observed symptoms of detriments of acid rain. Compared to controls (pH 6.5), pollen germination decreased by 41.75% at pH 3.3 and pollen tube elongation decreased by 24.3% at pH 3.4. Acid rain threshold proportion value was around pH 3.3 and 3.4 for apple pollen germination and pollen tube elongation, respectively. Furthermore, pollen tube elongation was determined to be more sensitive to acid rain than pollen germination. The pH values below 3.1 resulted in complete destruction of pollen tubes. Pollen germination entirely stopped at around pH 3.0. Finally, it has been shown that the acid rain has a blocking effect on pollen germination and pollen tube elongation in apple. The conclusion is that not only pH value but also the quantity of acid rain is important factor in germination. The results were found statistically significant through the LSD test at levels of $p < 0.05$ and $p < 0.01$.

Keywords: Acid rain – *Malus sylvestris* – pollen – germination – tube growth

INTRODUCTION

Health of humans, plants and animals depend on unpolluted atmosphere. The polluting elements such as SO_2, NO_X, CO_2 and HF, which are emitted to the air by the burn of fossil veins, lead acidic deposition (or acid rains as known) as a result of complex physical and chemical reactions. Sunlight accelerates most of these reactions. The transportation of compounds, which lead to acid rains, through the prevailing winds for thousands of miles raises the pollution to very high rates. The plants, the primary producers, are affected much from the pollution.

Studies on the effects of acid rain on plants have been performed mostly on vegetative organs, showing that acid rains affects vegetative organs of the macroscopic, microscopic and biochemical levels [6, 8, 11, 15, 17, 20].

Acid rains not only affect vegetative organs but also the generative structures of plants. Pollen is the first of these structures. It has been determined that SO_2 inhibit-

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ed pollen germination and pollen tube growth under laboratory conditions [4, 10, 12, 21]. Similar results have been reported for pollutants as acid rains [3], NO_x [16], HF [5] and exhaust gasses [9].

Considerable levels of acid rains have been determined in different parts of Turkey. Effects of these rains on the livings and non-livings have not been investigated sufficiently. The plans for new Thermic Power Plants in order to make the fossil veins economically productive, the broadening of traffic net and the increase on the number of the motor vehicles contribute to this harmful situation. It is important to find out the threshold values at which the acid rain will be harmful for a plant species/organs. In this study, the effects of simulated acid rain on pollen germination and pollen tube growth of apple have been investigated under laboratory conditions.

MATERIALS AND METHODS

In this study, pollens of apple flowers (*Malus sylvestris* Miller cv. Golden) were used as material. Flowers were placed in polyethylene containers and the experiments were done without any delay in laboratory. Flowers from the same tree were used in every sequence of experiment. Also, flowers were from same maturity level. H_2SO_4 (Merck) and deionised water was used for preparing simulated acid rain solutions. Acid rain is often defined as precipitation with a pH of less than 5.6 [14]. Thus, pH values of solutions were as 5.0, 4.8, 4.6, 4.4, 4.2, 4.0, 3.8, 3.6, 3.4, 3.3, 3.2, 3.1, 3.0 and 2.9. Deionised water (pH 6.5) has been used as control. pH measurements have been done with a digital pH meter (WTW 330).

Pollens were germinated in Brewbaker and Kwack [1] culture solution (culture medium) under various conditions: Firstly, pH solutions and culture medium at the same volumes were used. Three sterile micro slides were prepared for each pH value (2 for experiment, 1 for control). A 50 µl culture solution was dropped to 2 various areas on each slide. Fifty µl acid rain for experiment groups and 50 µl deionised water (pH 6.5) for control group (CG) were added onto slides. Pollens on anther were homogeneously cultivated by a sterile syringe into the culture medium under stereomicroscope [19]. Petri dishes (15 cm diameter) with a moist filter paper lining the lower plate served as an improvised humidity chamber. Two glass rods were placed parallel at about 4 cm apart on the moist filter paper to facilitate the handling of the pollen cultures. Then, the Petri dishes were settled in incubator at 22 ± 2 °C. Each germination medium was fixed with 10% ethyl alcohol after 3 hours [19], and then, lamellae were closed. Germination percentages and tube lengths of pollens were determined under light microscope by the method of Shivanna and Rangaswamy [19]. Meanwhile, pollens were germinated in only culture solution (CM, pH 6.5) or only in deionised water medium (pH 6.5) as controls.

Secondly, to determine whether acid rain duration has any effect on pollen germination rate two different exposure times (3 hours and 9 hours) were applied.

Thirdly, pH solutions and culture medium, at different volumes, were used because not only the acidity degree but also the period and quantity of the acid rain

are important factors. For this aim, pollens were germinated in 50 µl culture solution plus 100 µl acid rain in A series (deionised water instead of acid rain for control group = CG A) and 100 µl culture solution plus 50 µl acid rain in B series (deionised water instead of acid rain for control group = CG B).

All experiments were repeated three times and results were statistically analyzed by calculating variance and the standard error (Sx) of the mean. Statistical analysis was performed based on SPSS (version 10.0) program. In order to detect the significance of differences ($p < 0.01$ or $p < 0.05$) of variables, a multiple comparison (LSD) test was performed.

RESULTS

In the germination medium series which contained 50 µl culture solution and 50 µl simulated acid rain, pollen germination and tube length are given in Figures 1 and 2.

Pollen germination and pollen tube length did not cause important change ($p > 0.05$) between control (CG) and pH 3.6-solution treatment**.** Pollen germination at this range was determined as 88 ± 11% (pH 3.6) minimum and 94 ± 6% maximum (pH 5.0) (Figure 1). Length of pollen tube was measured as 619 ± 64 [pH 3.6 (maximum)] and 702 ± 84 µm [pH 4.2 (minimum)] (Figure 2). Although pollen germination was decreased 17.5% at pH 3.4 as compared to the control (CG), this was not statistically significant ($p > 0.05$). The germination was determined as 53 ± 5% at pH

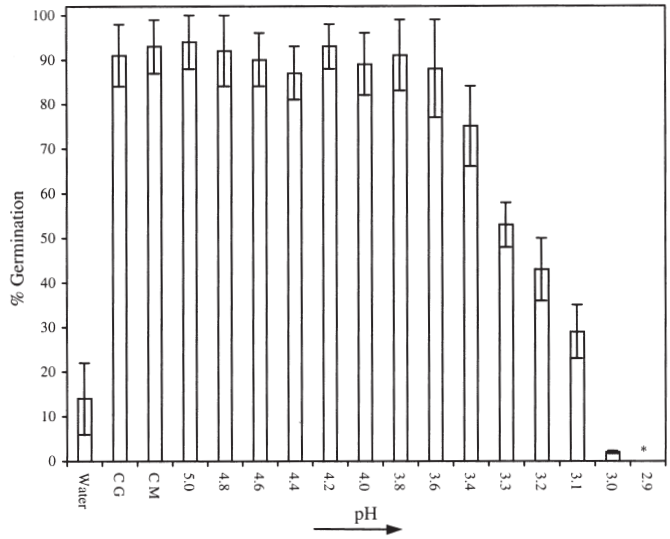

Fig. 1. Germination rates of pollens incubated for 3 h in an acid rain simulated medium (1 : 1 v/v). All values are averages of three independent experiments; standard errors are indicated as bars (*No germination)

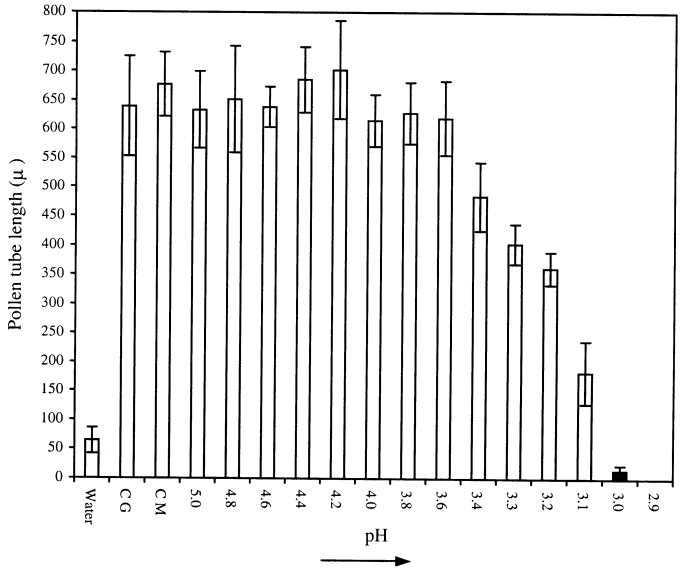

Fig. 2. Tube lengths of pollens incubated for 3 h in an acid rain simulated medium (1 : 1 v/v). All values are averages of three independent experiments; standard errors are indicated as bars (Black histograms: pollen tubes have been partly destroyed)

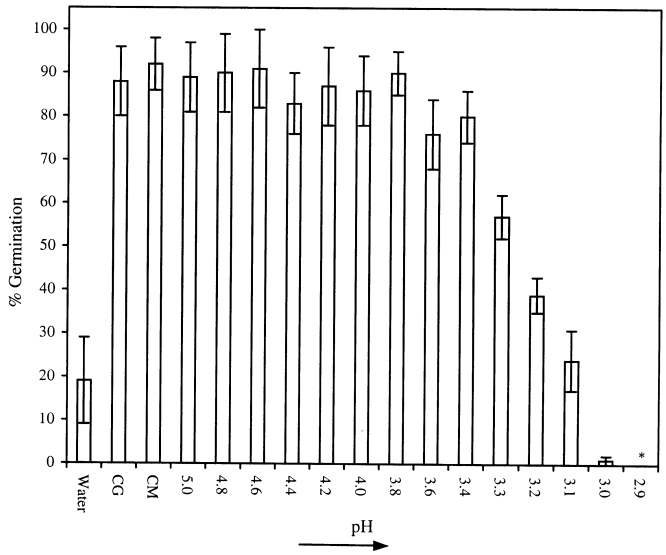

Fig. 3. Germination rates of pollens incubated for 9 h in an acid rain simulated medium (1 : 1 v/v). All values are averages of three independent experiments; standard errors are indicated as bars (*No germination)

3.3. This result depicted an important ($p < 0.01$) decrease as 41.75% compared to CG. Length of pollen tube was 484 ± 59 µm at pH 3.4 and 361 ± 28 µm at pH 3.3. These figures were statistically significant decreases compared to the control group (as 24.3% and 45.4% corresponding to a level of $p < 0.05$ and $p < 0.01$, respectively). Considerable decreases occurred for germination (starting from pH 3.3) and pollen tube length (starting from pH 3.4) depending on the increases at acidity degree. At pH 3.0, the germination rate was 2 ± 0.3% and the pollen tube length was 15 ± 8 µm (Figs 1 and 2). Some pollen tubes were, however, observed to be destroyed. The germination completely stopped at pH 2.9. Usage of culture solutions on germination medium did not cause an important change on germination rates of pollens ($p > 0.05$) (Fig. 1).

Results of the second series of experiments were different from first experiment series only in the length of incubation period (3 hours instead of 9 hours), are given in Figs 3 and 4.

The germination percentages of pollens at these series showed similar results to the first series. Considerable increases were observed for the pollen tube lengths for all groups. However, inhibitive effects of acidity degree on pollen germination and pollen tube elongation were in harmony with the first series of experiments. The only difference between the first and second series was that the acid rain started to inhibit pollen tube elongation at pH 3.3 not at pH 3.4 as in the first series. Elongation of pollen tube was decreased 37.5% at pH 3.3 compared to control (CG) ($p < 0.01$). Although this value was measured as 24.8% for pH 3.4, the value was not statistically significant ($p > 0.05$).

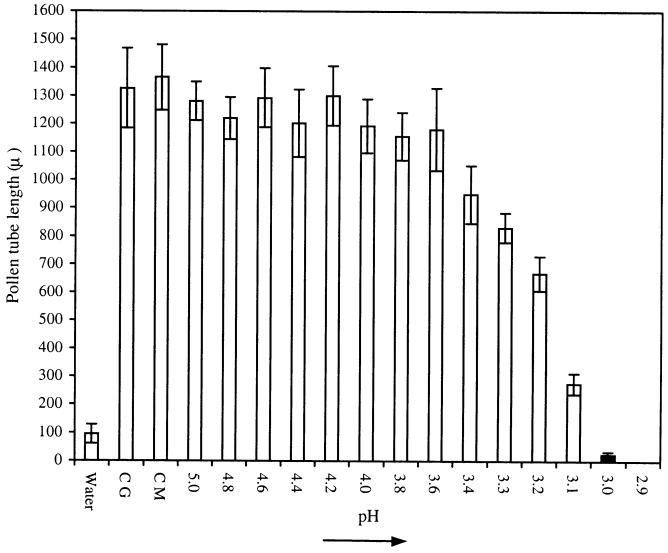

Fig. 4. Tube lengths of pollens incubated for 9 h in an acid rain simulated medium (1 : 1 v/v). All values are averages of three independent experiments; standard errors are indicated as bars (Black histograms: pollen tubes have been partly destroyed)

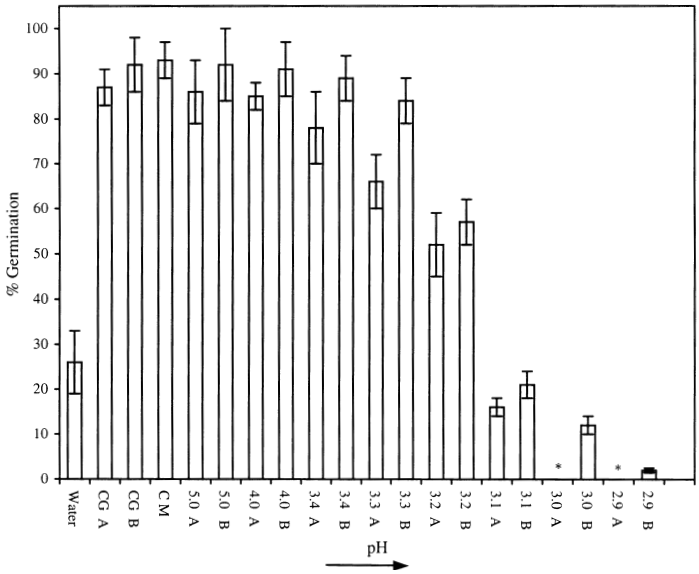

Fig. 5. Germination rates of pollens incubated for 9 h in an acid rain simulated medium (series A, 1 : 2 v/v; series B, 2 : 1 v/v). All values are averages of three independent experiments; standard errors are indicated as bars (*No germination)

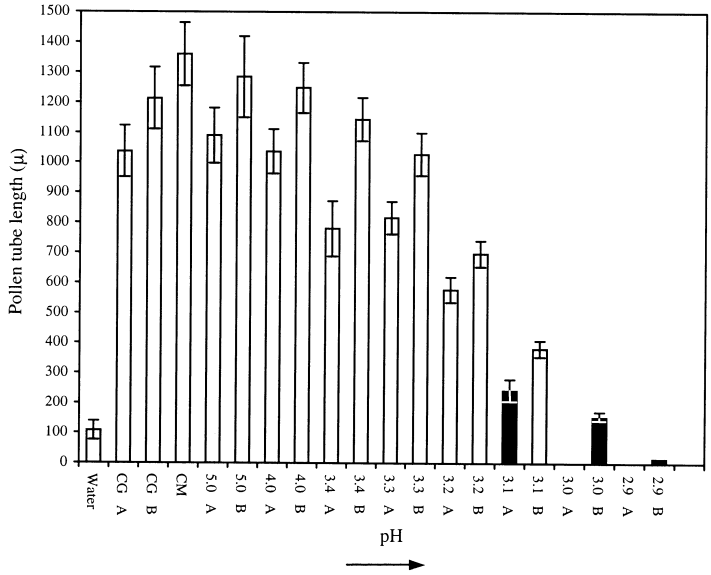

Fig. 6. Tube lengths of pollens incubated for 9 h in an acid rain simulated medium (series A, 1 : 2 v/v; series B, 2 : 1 v/v). All values are averages of three independent experiments; standard errors are indicated as bars (Black histograms: pollen tubes have been partly destroyed)

Results of experiment series with culture solutions and pH solutions for various volumes are given in Figs 5 and 6.

Pollen germination starting from pH 3.3 and pollen tube elongation starting from pH 3.4 were decreased at considerable rates in series A in which mixture of 50 µl culture solution and 100 µl acid rain was used ($p < 0.05$, $p < 0.01$). A decrease (40.3%) has been determined for germination at pH 3.3 compared to control (CG A) pH 3.4 (Fig. 5). Here, a statistically important ($p < 0.05$) inhibition of the pollen tube elongation in the rate of 24.8% was observed (Fig. 6). Pollen germination entirely stopped at pH 3.0 in A (Fig. 5). Threshold proportion values were determined as pH 3.2 for pollen germination and pH 3.3 for pollen tube length in B series in which blend of 100 µl culture solution and 50 µl acid rain were used. Germination at pH 3.2 in B was decreased 38.1% compared to control (CG B) ($p < 0.01$). Also, pollens were germinated in very low rates ($2 \pm 0.5\%$) at pH 2.9 in B (Fig. 5). Pollen tube length was decreased 17.1% at pH 3.3 in B, a statistically important ($p < 0.05$) value (Fig. 6). Furthermore, pollen tubes were destroyed partly at pH 3.1 in A and mostly at pH 3.0 in B. Generally, lengths of pollen tube were different in series A and B. For example, pollen tube length at pH 4.0 in A was 17% less than pH 4.0 in B.

DISCUSSION

Sexual reproduction in flowering plants requires germination of pollens on stigmatic tissues. The pollens that germinate spontaneously on the stigma are particularly exposed to environmental stress. The pollen germination and pollen tube elongation are among the more sensitive botanical indicators of atmospheric pollution [2]. Decrease of liveliness rate on pollen and limitation of germination power, which are sensitive to pollutants affect the seed production ability of plant.

The results obtained from this study are that;

Firstly, pollen germination and pollen tube elongation in apple demonstrates an inhibition by acid rain. Various studies [18, 22] have reported this subject for pollens of different plant species. Decrease of pollen germination and impediment of pollen tube growth by acid rain stress may diminish the effect of pollination and fertilization and finally it may change quality and quantity of seeds.

Secondly, the threshold proportion level for apple pollen germination and pollen tube elongation is around pH 3.3 and pH 3.4. Furthermore, it suggests that the pollen tube elongation is a little more sensitive to acidity. Threshold proportion values, which lead the observed symptoms of detriments of acid rain, differ depending on the plant species and varied among the organs of the same plant. But the reports on this subject are usually on vegetative organs as leaf, young branch and root of plants [8].

Thirdly, pollen germination and pollen tube elongation on apple have been prevented by acid rain and this effect would not appear in a delaying or inhibiting manner. Because inhibitive effect of acid rain on germination and pollen tube elongation did not show a proportional decrease parallel to the lengthening of incubation peri-

od. This result is compatible with the observations of Renzoni and Viegi [13] on Pinus pollens.

Fourthly, it is concluded that not only the acidity but also the quantity of acid rain is important. Usage of culture solution and acid rain in the ratio of 1 : 2 or 2 : 1 in germination medium has caused differences on germination percentage and tube elongation.

Finally, the findings indicate that pH values below 3.1 damaged morphologies of pollen tubes and resulted in destruction of them and the germination of apple pollen entirely stopped at around pH 3.0–pH 2.9.

Causes of decreases of pollen germination and tube growth as a result of acid rain are unknown. But, it has been suggested that this reduction may be due to chemical or physical effects of acid rain on the stigmatic surface that result in creation of inharmonious pollens with the environment, or to a direct effect of rain on pollen tube [22]. Moreover, it has been reported that permeability of pollens to sucrose was reduced by medium acidity [13]. Since the stress reduce productivity, before all else, the behaviors of plant species to acid stress, as many other stress factors, fall into the scope of the researchers investigating in this area. Clarification of this situation will contribute to the promotion of productivity and raise of strong plants. Also, it is crucial to know the threshold proportion value of the factors that lead to stress. Only by this way, sensitive and durable species can be determined. If the stress will be at normal level and last for a limited period, the detriment will be temporary. In these circumstances, the plant can convert into its previous healthy state. If the pH is below the threshold value and exposure lasts for a long time, the plant may not yield or may not seed or may not survive any longer.

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